In-Vitro Starch and NDF Digestibility Using Rumen Fluid from Control and Bovamine® Supplemented Cows

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ABSTRACT: Probiotics are commonly fed to dairy cattle to improve feed efficiency and increase milk yield. The objective of this study was to determine the effects of a probiotic, (Bovamine®; Nutrition Physiology Company, LLC. Guymon, OK) consisting of *Lactobacillus acidophilus* (LA) and *Propionibacterium freudenreichii* (PF), on the *in vitro* digestibility of starch and neutral detergent fiber (NDF). Starch and NDF are the primary carbohydrate sources in dairy cattle diets from which they receive energy. There have been mixed results in performance when cows were fed the PF plus LA probiotic in beef and dairy cattle. The hypothesis of this experiment was that the probiotic would increase *in-vitro* starch and NDF digestibilities. Four rumen-cannulated, primiparous Jersey cows were used in a 2x2 crossover design experiment. Two cows were fed the LA and PF probiotic as a top dress and two were fed a control (ground corn) top dress. Rumen fluid from the two cows in each treatment group was pooled, creating two sources of ruminal fluid. The fluid was filtered and combined with a buffer before being inoculated into tubes containing no feed, ground corn, or ground alfalfa. Tubes were incubated for 0, 2, 4, 8, 12, 36, or 48 hours in a 39° C water bath with corn and 0, 2, 4, 8, 12, 36, 48, or 96 hours with alfalfa hay. Dry matter disappearance was used to represent starch digestibility since corn is 75% starch. After incubation, the samples were dried and analyzed. There were no significant changes in dry matter disappearance for corn or alfalfa. There was also no difference in digestibility rate or ruminal degradation in corn or alfalfa dry matter. There was a significant decrease in ruminal degradation of NDF in the Bovamine® supplemented group; however, this result would need to be confirmed with further study. From this study, it can be concluded that further research would be needed to determine if Bovamine® has any effect in the rumen or if the effect is primarily in the hindgut.

Introduction

Ruminants have a variety of microorganisms in their rumen, which breakdown nutrients supplied in the diet to products that can be digested in the intestines. These microorganisms include bacteria, protozoa, and archaea. Although they are the smallest, bacteria are far more numerous than protozoa or archaea, accounting for approximately 50% of total microbial volume (Kung Jr., 2001). Many bacteria prefer to breakdown a specific nutrient, such as amylolytic bacteria prefer starch. With an adequate diet, these different bacterial populations are balanced. When an animal's microbial population becomes unbalanced, decreased feed efficiency or milk yield may occur.

Probiotics are live microorganisms that are beneficial to the host's microbial population. They are commonly fed to dairy cattle to improve feed efficiency and milk yield, as well as enhance the animal's immune response. Because of this, probiotics can decrease the cost of production and lessen the negative environmental impact of raising dairy cattle. This study examined the effect of a probiotic on starch and neutral detergent fiber (NDF) digestibility. Starch and NDF are the primary carbohydrate and energy sources in dairy cattle diets. NDF is composed of cellulose, hemicellulose, and an indigestible component, lignin.

The probiotic of interest in this study was Bovamine® (Nutrition Physiology Company, LLC, Guymon, Oklahoma). This probiotic contains two bacterial strains, *Lactobacillus acidphilus NP51* (LA) and *Propionibacterium freudenreichii NP24* (PF). It is a combination of a lactic acid producing and lactic acid utilizing bacteria (Elam et al., 2003). LA is a lactic acid producing bacteria commonly used in probiotics because it may lower the pH in the small intestine, inhibiting growth of pathogenic organisms (Kung Jr., 2001). PF is the lactic acid utilizing bacteria, which is included in probiotics because it converts lactic acid to propionic acid (Kung Jr., 2001). These two bacterial strains should be beneficial together because as LA produces more lactic acid, PF can convert some of that to propionic acid. Propionic acid in the rumen can be utilized for energy, improving the growth rate of the animal (Kung Jr., 2001). Both of these bacterial strains are thought to work in the small intestine.

There have been mixed results from past studies on the LA plus PF probiotic. A study by Elam et al. (2003) showed the LA plus PF probiotic did not significantly affect the performance of beef steers[1] These steers were fed a high starch diet, 92% concentrate, in the feedlot. This study showed a significant difference in fecal shedding of *Escherichia coli*. *E. coli* shedding was lower in the steers fed the probiotic. Another feedlot study by Vasconcelos et al. (2008), showed an increase in the gain-to-feed ratio of beef steers when fed a high starch, 92% concentrate diet. A study by Raeth-Knight et al. (2007) with midlactation Holstein dairy cattle showed that the LA plus PF probiotic had no effect on diet digestibility, rumen fermentation, or overall performance.

Objective and Hypothesis

This study aims to determine the effects of the LA plus PF probiotic on the microbial population's ability to digest starch and NDF in the rumen using an *in-vitro* method. It was hypothesized that cows supplemented with the LA plus PF probiotic would have less potentially digestible starch and NDF remaining *in vitro* at all time points, when compared to the control cows.

Materials and Methods

The *in-vitro* procedure was adapted from a paper by Piwonka and Firkins (1996). *In-vitro* studies were conducted anaerobically and at 39° C to simulate the conditions in the rumen of the cow. Gaseous microbial waste was released through a one-way valve in the stopper.

Four rumen-cannulated, primiparous Jersey cows at the Waterman Dairy Center (Institutional Animal Care and Use Committee approved; Protocol No. 2015A00000047) were used in a 2x2 crossover design experiment. The diet was 36.8% corn silage, 17.2% ground corn, 13.8% alfalfa balage, and also contained protein and energy supplements, vitamins, minerals, and amino acids. There were no probiotics in the diet. Two cows were fed the LA plus PF probiotic as a top dress and two were fed a control (ground corn) top dress.

Collection: All four cows were given the ground corn top dress and the new diet during a two-week covariate period. The treatment cows received the probiotic during the experimental period, which was four weeks and ruminal fluid was taken at the end of the third week. After the first experimental period, all four cows were given ground corn as a top dress during the two-week washout period. For the next experimental period, the cows that originally were receiving the probiotic were now given the control top dress. The cows that had received the control top dress were switched to the Bovamine® top dress. Samples were again taken at the end of the third week of the experimental period. Rumen fluid from each cow was collected and strained through four layers of cheesecloth at the farm to remove large solid particles.

Sample Preparation: Once back at the lab, rumen fluid from the two cows in each treatment group was pooled, creating two sources of ruminal fluid. The sample was blended for one minute in a blender, and then strained through eight layers of cheesecloth twice to remove as much solid as possible. The rumen fluid was anaerobically combined with a simplex buffer (**Table 1)** at a 1:1 ratio. To maintain the anaerobic environment, each piece of glassware used was flushed before and during use with carbon dioxide.

Table 1. Simplex buffer composition (Piwonka and Firkins, 1996)

11.43 g **K_2HPO_4** 9.0 g **KH_2PO_4** 1.17 g **NaCl** 0.162 g **$MgSO_4 \cdot 7H_2O$** 18 drops **0.1% Rezazurin** 2.1 L **Distilled H_2O** 13.5 g **$NaHCO_3$*** 19.8 mL **2% L-Cysteine HCl****	*Solution was flushed with CO_2 through a bubbler and boiled for 30 minutes. After cooling, the $NaHCO_3$ was added and the solution was bubbled until the color changed to pink. ** When the solution was pink, the 2% L-Cysteine HCl was added and bubbled until the solution was colorless. Then, the solution was autoclaved.

The 40 mL centrifuge tube contained either no feed, ground corn, or ground alfalfa. Each tube was inoculated with 30 mL of the simplex buffer and rumen fluid mixture. **Table 2** shows the contents of the tubes for each treatment. Next, they were capped with a stopper that contained a one-way valve to allow for gases to be released but no oxygen to enter. At each time point, there were three tubes for each treatment group.

Incubation: The individual tubes were inverted and incubated for 0, 2, 4, 8, 12, 36, 48, or 96 hours in a 39° C shaking water bath. Twice daily, the tubes were inverted to distribute the feed particles throughout the tube. After incubation, the samples were immediately placed on ice to stop fermentation. After fermentation had stopped, the samples were refrigerated, centrifuged, and dried.

Table 2. Treatment descriptions for the starch and NDF digestibilities *in-vitro*

Tubes for Starch Analysis

Control (1)	Fed Control (2)	Bovamine® (3)	Fed Bovamine® (4)
• 30 mL of control rumen fluid + simplex buffer mixture • Unfed	• 30 mL of control rumen fluid + simplex buffer mixture • 0.5 g ground corn	• 30 mL of Bovamine® rumen fluid + simplex buffer mixture • Unfed	• 30 mL of Bovamine® rumen fluid + simplex buffer mixture • 0.5 g ground corn
*All tubes were inoculated with a mixture of rumen fluid and simplex buffer at a 1:1 ratio. ** Tubes used to analyze starch digestibility were incubated up to 48 hours.			

Tubes for NDF Analysis

Control (5)	Fed Control (6)	Bovamine® (7)	Fed Bovamine® (8)
• 30 mL of control rumen fluid + simplex buffer mixture • Unfed	• 30 mL of control rumen fluid + simplex buffer mixture • 0.5 g ground alfalfa	• 30 mL of Bovamine® rumen fluid + simplex buffer mixture • Unfed	• 30 mL of Bovamine® rumen fluid + simplex buffer mixture • 0.5 g ground alfalfa
*All tubes were inoculated with a mixture of rumen fluid and simplex buffer at a 1:1 ratio. ** Tubes used to analyze NDF digestibility were incubated up to 96 hours.			

Analysis: The tubes from the same treatment were combined into one representative sample for each time point. The NDF content of the ground alfalfa and residues from tubes 5, 6, 7, and 8 at the respective time points was determined using the Ankom Fiber Analyzer (Ankom Technology, Macedon, NY) using the procedure outlined by Van Soest et al. (1991). The ground alfalfa was determined to contain 43.3% NDF. Because ground corn is approximately 75% starch on a dry matter basis, starch content was expected to decrease at the same rate as dry matter. Therefore, tubes from treatments 1, 2, 3, and 4 were only analyzed for changes in dry matter remaining.

Data Analysis: Kinetics of dry matter disappearance was done using the log-linear procedure to obtain initial parameter estimates. Iterations using the nonlinear procedure in SAS[6] using the initation estimates determined for the potentially digestible fraction, rate of digestion, and an indigestible fraction. A rate of passage of 0.05/hour was used to calculate ruminal degradation. This rate is standard for a lactating cow eating approximately 22 kg/day of dry matter. SAS was also used to determine significance between control and treatment groups using the two *in-vitro* replications.

Results

To determine changes in dry matter digestibility over time, the sample was split into A, B, and C pools. The A pool was the rapidly degraded fraction, B pool was the slowly degraded fraction, and C pool was the indigestible fraction. The digestibility rate (Kd) and ruminal degradation (RD) were also determined. The A and B pools, as well as the digestibility rate, were used to calculate the percentage of ruminal degradation. The C pool is not used because it is the indigestible component, and therefore, cannot be degraded in the rumen. **Table 3** summarizes the results for corn dry matter (DM) and alfalfa DM and NDF. Corn DM showed no significant differences between the control and Bovamine® supplemented groups, although slightly higher percentages of rapidly digested DM and RD were noted for Bovamine®. No significant differences were observed between the two groups in the DM from the samples containing alfalfa. In the alfalfa samples, RD of NDF was lower for Bovamine®. This significance should be questioned because of variability of the data and the exceptionally high Kd. In all of the tubes containing alfalfa, the results for Kd were inconsistent with the expected result. The digestibility rate should be higher for tubes with corn than for fiber, but the Kd values for fiber are erroneously higher. Fiber spends more time in the rumen to be broken down by the bacteria. These results show Kd values for alfalfa, 0.1414 to 0.1950/hour that were more than double that of the values seen in the tubes with corn, 0.0536 and 0.0568/hour, which are reasonable estimates. Because of this variation from what should have been seen and the digestive physiology of a cow, it would be challenging to draw any conclusion from the significant decrease in RD in the Bovamine® treated group without further research.

Table 3. Least squares means of the kinetics of DM digestibility of corn and alfalfa and NDF digestibility of alfalfa in vitro

Item		A Pool (%)	B Pool (%)	C Pool (%)	Kd (/h)	RD[1] (%)
Corn						
Control	DM	7.5	62.2	30.3	0.0536	39.4
Bovamine	DM	14.0	56.6	29.4	0.0568	42.7
Alfalfa						
Control	DM	11.2	25.2	63.5	0.1415	28.4
Bovamine	DM	9.6	24.7	65.7	0.1950	28.3
Control	NDF	8.8	30.3	61.0	0.1614	31.9[a]
Bovamine	NDF	7.8	60.5	31.8	0.0453	22.0[b]

[1]A rate of passage of 0.05/hour was assumed.
[ab]Least squares means in same column within plant component differ.

As expected, **Figure 1** shows that over time the amount of corn remaining decreased in both the control and probiotic supplemented groups. There was no significant difference between the two groups. Both treatments decreased the percent of DM remaining until approximately 40% was remaining after 48 hours. **Figure 2**, which shows the percentage of DM remaining per hour in the tubes containing alfalfa, also showed a decrease in DM remaining over time as expected. The Bovamine® supplemented group appeared to have less DM remaining at each time point, but it was not statistically significant **(Table 1).** Dry matter remaining decreased in the tubes containing alfalfa until approximately 70% was remaining at 36 hours and was consistent at 48 and 96 hours. **Figure 3** shows the percent of NDF remaining per hour in the tubes containing alfalfa. The percent remaining decreased to approximately 65%, but fluctuated more than expected, likely due to error. One of the major problems with the study was a small number of samples. There were only two treatment and two control samples. Although each sample was pooled from two cows to decrease variability, this did not eliminate the problem. The second problem was that the tubes only contained 30 mL of fluid and no feed or 0.5 grams of feed. After drying the samples, there was only a small amount of sample in each tube that could be used for analysis. Even after combining the three samples for each time point, there was barely enough sample in the unfed tubes to run the analysis in duplicate. The test could not be repeated, even when the coefficient of variance values from the first run indicated that the test should be re-run.

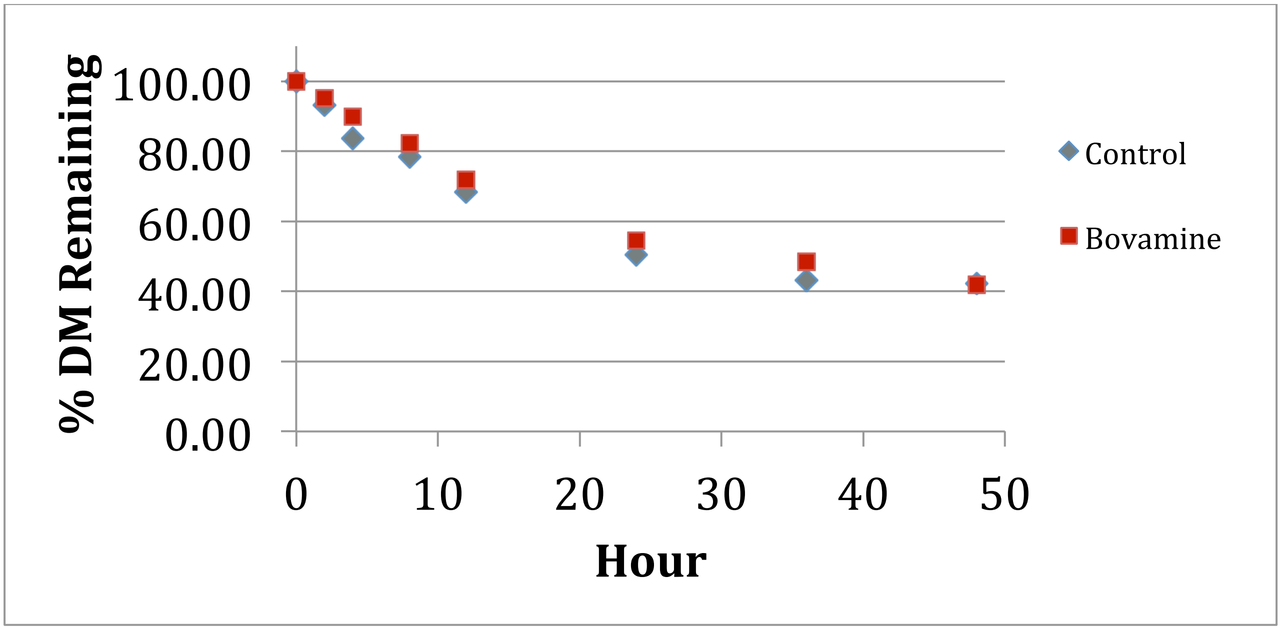

Figure 1. Percentage of DM remaining per hour in the tubes containing corn

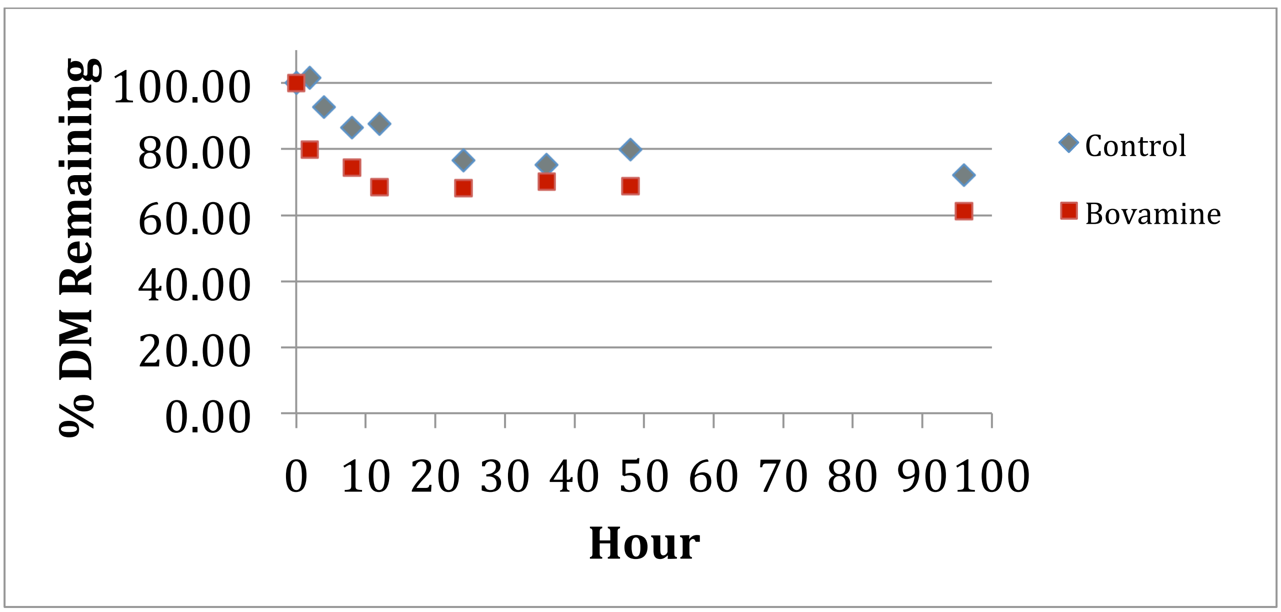

Figure 2. Percentage of DM remaining per hour in the tubes containing alfalfa

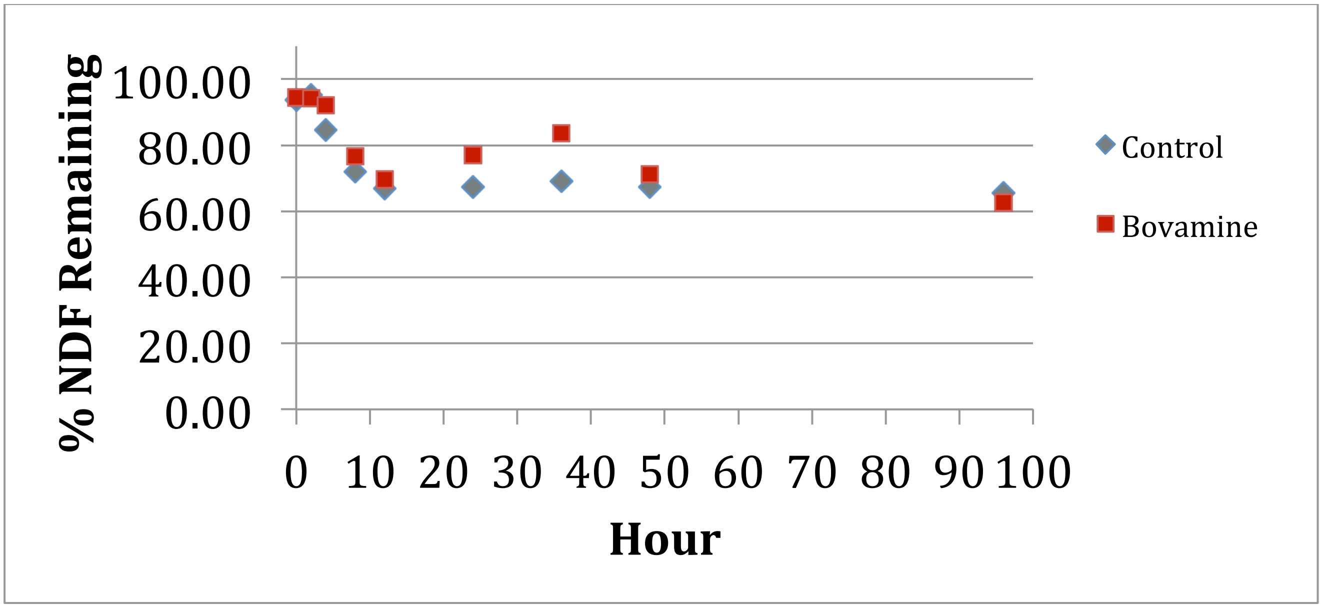

Figure 3. Percentage of NDF remaining per hour in the tubes containing alfalfa

Conclusions

The importance of this study is that it aimed to provide insight into the effect of the probiotic on ruminal digestibility in the cow. An effective probiotic can increase feed efficiency, which decreases cost of production and lessens the negative environmental impact of raising cattle. There was no significant difference *in-vitro* between the dry matter disappearance of corn in the rumen fluid from control and LA plus PF probiotic supplemented cows. There were similar results for alfalfa dry matter and NDF digestibilities. There was a significant decrease in ruminal degradation of NDF in cows treated with the probiotic. This finding is likely due to error and further research would be needed to confirm the results. The results of this study suggest that the effect of this LA plus PF probiotic may not be in the rumen, but its effects could be in the hindgut. Further research measuring ruminal and total tract digestibilities may be a better indicator of the effectiveness of the probiotic.

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